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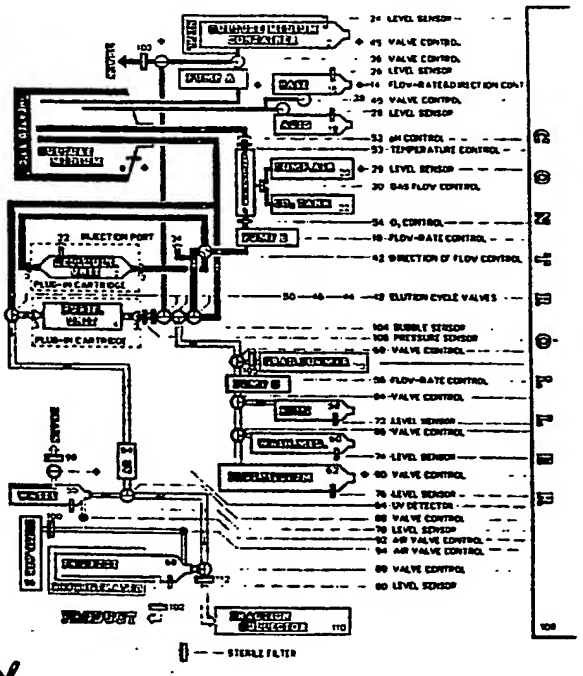
(54) Title: AN INTEGRATED CELL CULTURE-PROTEIN PURIFICATION SYSTEM FOR THE AUTOMATED PRODUCTION AND PURIFICATION OF CELL CULTURE PRODUCTS

(57) Abstract

An integrated cell culture-protein purification system has been developed for the continuous, automated production of pure cell culture protein products. The instrument comprises a bioreactor subunit (2) having a hollow fiber bioreactor to culture and maintain cells which secrete the desired product into the cell culture medium. The culture medium is circulated through a purification cartridge (4) which adsorbs the desired product. The system is capable of continuously removing the product from the culture medium by immunoaffinity adsorption and pure product is then recovered. The product can also be recovered automatically in a discontinuous operation from the spent culture medium by either affinity, ion exchange, or hydrophobic purification techniques. The integrated system allows continuous production of high-quality pure proteins from cell culture.



The Integrated Cell Culture Purification System

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An Integrated Cell Culture-Protein Purification System for the Automated Production and Purification of Cell Culture Products

Background

Complex proteins are increasingly used in research, diagnostics and therapeutics. Many of these proteins can only be produced in appropriate eucaryotic cells. With the advent of hybridoma technology and other progress in genetic engineering of eucaryotic cells, mammalian or yeast cell lines are becoming the method of choice for producing complex proteins on a large scale.

The secreted product needs to be purified from the cell culture medium. Most mammalian cells require serum which contains a diverse mixture of proteins, many of which are present at high concentrations. Even in serum-free media systems, numerous other proteins are secreted from the cells. For most of the applications the final product has to meet high levels of purity and activity.

The successful production of these proteins depends largely on the development of fast and efficient methods of purification. Typically, the purification constitutes the major cost (up to 80% of the total cost) in these processes. The large scale use of these protein products is hindered because of the high cost.

There is an urgent need for processes to produce proteins in a simple and economical way. Significant cost reduction in the production of protein biologics could be realized if the purification would be integrated with cell culture into a fully automated system. In addition, the product quality is also expected to improve because the secreted protein is continuously removed from the culture medium in which the product is exposed to catabolic enzymes. Protein identity is an important issue for protein biotherapeutics, i.e. the final product should be free of degraded or other aberrant protein molecules. The integration requires that the presence of the purification unit in the cell culture system would not affect the conditions of cell culture. Therefore, highly-specific purification methods like affinity/immunoaffinity

chromatography is needed to make the integration of cell culture and purification feasible for the continuous purification of secreted product.

Progress in cell culture technology has led to the development of membrane bioreactors for growing eucaryotic, such as mammalian cells within well-defined compartments. Cells grown inside low nonspecific adsorption flat sheet or hollow fiber membranes in thin (200-400 um) layers are continuously perfused with nutrients and grow to cell densities previously unattainable by the stationary, stirred tank or airlift-type fermentors. Nutrient deprivation or shear sensitivity issues are minimized by this technology. This allows high cell viability in the bioreactor and minimize DNA contamination of the product. The microfiltration membrane eliminates the opportunity of bacterial contamination of the bioreactor. The cells are grown at tissue density with high production rates surpassing the production capacity of conventional bioreactors. After populating the available compartment space, the cells reach a growth-arrested state in which most of their energy is directed towards production. This configuration allows the highest production capacity per unit volume of bioreactor space.

Another important aspect of the integration is the availability of appropriate protein separation technologies. Current protein purification technologies require significant improvement in order to realize the potentials of the integration concept. A major obstacle is that the interaction of the cell culture medium with the protein separation material (chromatography resin) may change the composition of the medium which can be detrimental to the cells in culture. Chromatography media like ion exchange or hydrophobic matrices can drastically change the culture medium composition and thus are unsuitable for an integrated instrument if continuous removal of the product is desired. Biospecific, affinity separation is the only method offering the least interference with cell culture. However, current affinity technologies have serious shortcomings which have prevented them from being incoporated into an integrated system.

The integration of cell culture with continuous purification of secreted product without jeopardizing the cell culture by introducing potentially toxic

chemicals and bacterial/viral contamination necessitates the development of a stable, nontoxic, chemically inert, sterilizable activated affinity chromatography resin. Current activated affinity matrices cannot be incorporated into the integrated instrument because they do not meet the criteria of being chemically inert, nontoxic, stable, and sterilizable. The most commonly used coupling methods employ reactive electrophilic centers with leaving group displaced by the incoming nucleophilic ligand (protein/antibody). These displacement reactions frequently remain incomplete even after capping the unreacted sites and continue to release leaving groups, many of them are toxic to cells. Conversely, constituents of the culture medium may be covalently attached to the matrix. The affinity resin may leach other toxic molecules, like isocyanate from CNBr-activated matrices, for long periods of time. This is toxic to the cells in the bioreactor. The immobilization method may also increase the protease sensitivity of immobilized protein (antibody) ligand, an issue which is a problem with the traditional coupling chemistries.

In affinity separations, proteins (antibodies) are frequently used as ligand. In the integrated system, the immunoaffinity chromatography resin must withstand the conditions of cell culture for long periods of time. The warm, highly-oxygenated environment of cell culture medium may diminish the activity of the immunoaffinity column. Many of the cultured mammalian cells, including hybridomas, secrete proteolytic enzymes which may degrade the immobilized antibody ligand. The same applies to dead cells spilling their content into the culture medium. The structure of the support and the method of immobilization also plays an important role in the protease sensitivity of immobilized antibody. The low concentration of secreted proteins in the cell culture medium may also complicate quantitative recovery of the product.

Current immunoaffinity technologies make process automation complicated because of the continuous loss of immunosorbent capacity. This is the result of ligand leaching and inactivation of immobilized antibody for reasons mentioned above. The total cycle life of the immunoadsorbent (5-30 cycles) is usually too short to make this technology suitable for the integration of bioreactor and purification for continuous product recovery. All these issues need to be addressed in order to make affinity chromatography media compatible with mammalian cell culture.

Description of the Drawings

Figure 1. is the flow diagram of the invention integrated cell culture/purification system.

Figure 2. is the accumulated production of purified monoclonal antibody in the integrated cell culture/purification system.

List of Reference Numerals

2 Cell culture unit/bioreactor
4 Purification unit/chromatography cartridge
6 Oxygenator
8 Cell culture medium vessel
10 Pump B
12 Cell culture medium container
14 Pump A
16 Base container
18 Acid container
20 Compressed air source
22 Carbon dioxyde source
24, 26, 28 29 Level sensors
30 Gas flow control
32, 34 Injection ports
36 Three-way valve
38, 40 Two-way valves
42 Four-way valve
44, 45, 46, 48, 50 Three-way valves
52 pH probe
53 Temperature probe
54 Dissolved oxygen probe
56 Pump C
58 Culture medium container
60 Wash medium container
62 Elution medium container
64 Flow cell UV monitor

66 Waste fluid container
68 Product vessel
70 Gradient former
72, 74, 76, 78, 80 Level sensors
82, 84, 86, 88, 89 Three-way valves
90 Two-way valve
92, 94 Two-way air valves
96 Compressed air source
98, 100, 102, 103, 105, 112 Sterile filters
104 Pressure sensor
106 Bubble sensor
108 Controller
110 Fraction collector

Description of the Preferred Embodiment

Cell culture is a versatile technique for producing a variety of complex biomolecules including proteins. The protein of interest is produced and secreted by the cultured cells into the cell culture fluid of which the product is recovered, in many cases, by using complicated, multi-step purification procedures. This frequently results in significant product losses and an increased possibility of generating aberrant protein molecules. We have developed a new integrated instrument which unifies the formerly separate cell culture and protein purification into an integrated, automated operation which significantly reduces the manufacturing cost of high purity cell culture protein products. The product quality is also increased as a result of the integrated production of proteins. The integrated cell culture/purification instrument has two subunits, such as the bioreactor and chromatography subunits (Fig. 1).

In the subject invention, the cell culture unit is a hydrophilic hollow fiber bioreactor (Zymax, Microgon). The 2 bioreactor consists of compact coaxial fibers in a cylindrical housing. The fibers are usually 0.2-1.0 mm in diameter with a pore size of 0.2 micron. The space within the fibers is called intra-capillary space (ICS) and the outside region is designated extra-capillary space (ECS). The cells are detained in ECS whereas ICS has nutrient or culture medium flowing through. The pore size of the fibers is small enough to contain the cells (approx. 10 microns in diameter), but allows exchange of nutrients and proteins across the membrane by diffusion.

The bioreactor environment needs to be carefully controlled because slight changes may lead to decreased productivity or cause cell death. The important parameters to be controlled are temperature, pH, dissolved oxygen and nutrient levels of the culture medium. Mammalian cells are very sensitive to chemical contaminants. A wide variety of substances even at ppm level could be highly toxic to the cells. Under the right culture conditions the cells remain active in the bioreactor for a couple of months or even longer.

To control bioreactor conditions, probes, such as the 53 temperature probe, 52 pH electrode and 54 dissolved oxygen probe need to be included into the bioreactor loop and linked to the programable 108 controller. A suitable controller may be a microprocessor controlled unit like the Proteus 2000 (Wheaton Scientific Instruments). The 108 controller receives information from the probes and makes appropriate adjustments in the above culture conditions. The pH is adjusted by autotitration of the culture medium from 18 acid or 16 base containers. The temperature is adjusted by warming the medium flask while the dissolved oxygen is changed by a 30 gas flow controller by increasing the air pressure on the 6 oxygenator.

The 6 oxygenator (Microgon) is also of hollow fiber-type containing hydrophobic fibers with a pore size of 0.02 micron; 5% CO_2 in air mixture is passed through the oxygenator. The bioreactor loop contains the culture medium supply system which, in the simplest case, is a medium container changed periodically as the medium gets exhausted. In an automated fashion, feeding of cells is accomplished by continuous perfusion (feed and bleed system) by introducing fresh and withdrawing spent medium from the bioreactor loop at a preset rate. This is accomplished by using the 14 reversible pump (Pump A) which first withdraws spent medium from the 12 medium vessel and then replenishes it with fresh medium. The spent medium exits the system through the 45 valve and 103 hollow fiber filter, 0.2 micron, into the drain. Alternatively, the spent medium may be recycled through 45 and 46 valves onto the 4 purification unit for subsequent purification of the product.

The 2 bioreactor and 4 affinity chromatography column is enclosed into plug-in type cartridges which are presterilized and attached to the instrument through 3 snap-in connectors. After each run, the cartridges are discarded. The rest of the instrument can be sterilized in place by using a chemical sterilant, such as 5% glutaraldehyde. After the specified time of sterilization, the glutaraldehyde is drained and the whole system is extensively washed with sterile, pyrogen-free deionized water (tissue culture grade) introduced through the connecting port of 70 gradient former. The bioreactor loop is then filled with culture medium and the 2 bioreactor is inoculated with the cells. The nutrients are delivered to the cells in the 2 bioreactor at a flow rate of 30-150 ml/min by using 10 Pump B.

The bioreactor loop contains 32 and 34 aseptic injection ports. Through 32 port, either medium can be withdrawn from the bioreactor loop or compounds can be introduced into the cell culture medium. Through 34 port, the bioreactor is inoculated with the cells to start operation. The 42 four-way valve and the 48 and 50 three-way valves direct the culture medium on or off the 4 purification unit. The 4 purification unit is not in operation before the cells populate the bioreactor. In the continuous mode of operation, after the sixth day, the 108 controller initiates the product recovery cycles.

The bioreactor was inoculated with $5x10^7$ hybridoma cells. The mouse x mouse hybridoma (HB 57) producing IgG1 monoclonal antibody was grown in RPMI 1640 medium supplemented with antibiotic solution and 10% iron-supplemented calf serum. This hybridoma is a low producer and requires a minimum of 10% serum for optimal growth and antibody production. The culture medium is kept at 37 C in the bioreactor loop. Cell density and viability were determined by using hemocytometer and Trypan blue staining.

The bioreactor loop interfaces with the purification/chromatography loop through the 4 purification unit which can be an affinity cartridge. The affinity cartridge is a polymeric cylindrical container closed with porous disks at the top and bottom and is filled with a fast flow activated affinity resin, Actigel-ALD Superflow (patent pending) to which an antibody to the desired product is attached. Actigel-ALD Superflow (Sterogene Bioseparations, Inc.) is a stable, nontoxic and sterilizable (autoclavable) activated resin. In this example, an affinity-purified, goat anti-mouse light chain-specific antibody is attached to the resin at a concentration of 10 mg/ml. The immunoaffinity resin has an extremely low content of leachables (< 0.1 ppm IgG) and is not toxic to mammalian cells. In the adsorption mode of operation, the culture medium is continuously circulated through the 4 affinity cartridge which adsorbs the monoclonal antibodies, secreted by the hybridomas in the 2 bioreactor, from the culture medium. Periodically, the 4 affinity cartridge is taken off-line from the bioreactor loop by using the 42, 48 and 50 valves and the product recovery cycle is initated by the 108 controller.

The accumulated antibody production of the system is shown in Fig. 2. After an approximately 1 week lag period, the antibody level has exceeded 10 ug/ml in the culture medium and continouous separation of the product has commenced. Over a period of 60 days, an accumulated 800 mg of affinity-purified monoclonal antibody was recovered from the integrated system. The purity of antibody was tested by SDS-polyacrylamide gel electrophoresis. Single heavy and light chains were observed indicating a protein purity of approximately 99%. Importantly, no sign of degradation of antibody is found which underscores the significance of the integration concept, i.e., the continuous removal of the product fom the culture fluid in which the antibody is exposed to proteolytic enzymes deriving from the serum and dead cells. Therefore, besides improving the economy of the process, the product quality is also improved.

If proteins other than antibodies need to be purified in a continuous fashion from the cell culture medium, the operator has to immobilize his antibody to the product to 4 purification unit which, in this case, contains Actigel-ALD Superflow activated support. The antibody mixed with the coupling reagent is injected into 4 cartridge through 32 sterile port and the reaction is allowed to take place for 6 h (Sterogene Bioseparations, Inc., Actigel-ALD Superflow Technical Bulletin). Unbound protein is removed by washing with 60 wash medium and then with 58 cell culture medium to prepare the cartridge for the product adsorption cycle.

The major advantages of the continuous removal of the product from the cell culture medium are as follows:

1. Significant improvement in product quality. The immediate removal of the protein product from the cell culture minimizes the chances of product degradation.
2. Significant reduction in process development time. The cell culture process development can be minimized because there is no need to adapt the cells to low serum or defined media. Serum-containg culture medium can be used because the affinity purification of the product eliminates the concern as to the presence of serum proteins. The generic purification method, immunoaffinity chromatography significantly simplifies product recovery.

The separation loop is driven by 56 Pump C which delivers the 60 wash-, 62 elution-, and 58 regeneration media in the specified sequence. First, the residues of the tissue culture medium and nonspecifically adsorbed proteins are removed by extensive washing with the 60 wash medium, 0.3 M NaCl. This step is followed by the elution of monoclonal antibodies from the 4 affinity column by using 1 bed volume of 62 ActiSep Elution Medium (patent pending) over a period of 30 min. ActiSep (Sterogene Bioseparations, Inc.) is a nondenaturing elution medium allowing 100 or more cycles to be performed on immunoaffinity columns. The eluant retains the binding capacity of the immunoadsorbent during many cycles of operation as well as high bioactivity of the eluted product. Eluted protein peak is monitored by OD_{280} measurement in a flow cell UV photometer and the protein peak is integrated to quantitate eluted antibody. The product is collected into a 68 refrigerated storage bottle while the washes are collected into a separate 66 waste bottle. When all the elution medium is recovered from the affinity column, another wash with 0.3 M NaCl is initiated to remove traces of the eluant. This is followed by a wash with the 58 cell culture medium (RPMI 1640) to equilibrate the column for the subsequent product adsorption cycle.

The 66 waste and 68 product bottles are emptied by 96 compressed air introduced through 100 sterile filter. The air flow is directed by the 92 and 94 valves into the respective bottles. The waste exits the system into the drain through 98 sterile filter, 0.2 micron pore size, and 90 two-way valve. The product exits the system through the 102 sterile filter, 0.2 um pore size, into a collection flask from which it is collected by the operator.

The emptying of the waste and product vessels is initiated by the 108 controller receiving signals from the 78 and 80 level sensors. The 72, 74, and 76 level sensors monitor fluid levels in the 56, 58, 60 and 62 buffer vessels. The 108 controller issues warning signals to the operator to fill empty bottles. Until the buffer/medium bottles are replenished, no further product adsorption and elution cycles are initiated by the controller. The separation loop contains additional safety controls, such as 104 pressure and 106 bubble sensors to protect the 4 purification unit from pressure build-up or from running dry.

3. Significant reduction in the manufacturing cost of cell culture products. The cost of cell culture can be reduced by a factor of 5-10 because there is no need to use defined media or expensive fetal bovine serum. The specificity of immunoaffinity separation eliminates contaminants of bovine serum origin; therefore there is no need for low IgG fetal serum. The cost of protein purification is also reduced by a factor of 10-15 because of the automation and long-term utilization of the immunoadsorbent.

Because of these advantages, continuous product recovery is strongly preferred. However, in certain cases when the characteristics of the product justifies it, discontinuous product recovery by other methods may be used. If the secreted product is resistant to proteolysis/degradation and the protein concentration in the cell culture medium is low, i.e. a low protein, defined medium is used, the product may be recovered from the spent culture medium in a discontinuous fashion as follows:

1. The spent medium, withdrawn from the 8 medium vessel by 11 reversible pump (Pump A) is directed by 45 and 46 three-way valves onto 4 separation unit/chromatography cartridge which may contain an ion exchange-, hydrophobic interaction(HIC)-, or affinity chromatography medium. In the event, if ion exchange chromatography is used, the column may contain e.g. a DEAE-type medium, equilibrated with 50 mM Tris, pH 8.0, delivered from vessel 62. Vessel 60 is filled with 50 mM Tris, pH 8.0, 1 M NaCl regeneration buffer. Vessel 58 contains a 2 mM Tris, pH 8.0 dilution buffer delivered by 56 pump (Pump C) to 4 chromatography cartridge along with the culture medium to lower the ionic strength of the cell culture medium. The appropriately diluted cell culture medium is applied to 4 ion exchange cartridge and washed with the 62 equilibration buffer. The desired product is then eluted by a gradient elution, generated by 70 gradient former from 0% (equilibration buffer) to 100% (end buffer, such as 50 mM Tris, pH 8.0, 0.3 M NaCl). The eluted product is measured at 280 nm in the 64 flow cell UV. monitor. The protein concentration is calculated by integrating the elution peak. Through the 89 three-way valve and 112 sterile filter, 0.2 micron pore size, eluted product exits the system into

the 110 fraction collector. The column is regenerated for the next adsorption cycle by washing with the regeneration buffer (vessel 60) and equilibration buffer (vessel 62).

2. In the event if HIC is preferred for the purification of the product, the 4 cartridge contains an appropriate medium, such as phenyl-, octyl-, butyl-, hexyl-, isopentyl-liganded or other HIC media. The 4 HIC cartridge is equilibrated with the binding salt by using 70 gradient former. The cell culture medium is then applied as described for the ion exchange chromatography separations. The binding salt is applied through 70 gradient former at the appropriate ratio. Unbound materials are washed with the binding salt solution and then product is recovered by applying a low salt buffer or a reverse salt gradient onto the 4 cartridge through the 70 gradient former. Through the 89 three-way valve and 112 sterile filter, 0.2 micron pore size, eluted product exits the system into the 110 fraction collector. The column is regenerated for the next adsorption cycle by washing with the regeneration buffer (vessel 60) and equilibration buffer (70 gradient former).

3. In the discontinuous product recovery mode, affinity chromatography may also be used. For example, 4 purification unit may contain immobilized Protein A or Protein G capable of binding antibodies. The culture medium is applied to the 4 affinity cartridge in accord with paragraph 1. A wash fluid, e.g. 50 mM Tris, pH 8.0, 0.15 M NaCl is then applied from vessel 60 and directed to 66 waste bottle. The lenght of the wash is determined by monitoring OD_{280} in the waste fluid. The bound antibody is then eluted by delivering one column volume of ActiSep Elution Medium from vessel 62 over a period of 30 min onto the 4 cartridge. If pH gradient elution is desired, 70 gradient former can generate the required pH gradient profile. Through the 89 three-way valve and 112 sterile filter, 0.2 micron pore size, eluted product exits the system into the 110 fraction collector. The column is regenerated for the next adsorption cycle by washing with the equilibration buffer (60 vessel).

Besides operating as a cell culture/purification system, the instrument can also operate as a stand alone cell culture unit to culture and characterize cell lines. This is important if a new cell line need to be developed and culture conditions optimized for the production of a particular protein.

When the cell culture conditions are optimized, an optimal purification strategy can be developed for the product which may be a continuous immunoaffinity method or a discontinuous conventional, ion exchange or HIC purification or some other affinity methods. This decision is made based on the cell culture conditions and the sensitivity of the product to degradation as well as the intended use of the protein. For therapeutic applications where the product identity is a major issue, continuous product purification is desirable as this method protects the product against degradation. If the product is more resistant to degradation and protein identity is of a lesser problem, discontinuous product purification may be suitable.

If purification of the secreted product is not desired, the separation loop can be utilized, independently from the bioreactor loop, for the purification of proteins like antibodies from biological fluids, such as serum or ascitic fluid. For the purification, ion exchange, HIC or affinity methods may be used following the description of paragraphs 1, 2 and 3. In general, the sample to be purified is applied to 4 purification unit from 58 vessel. This is followed by the application of the wash fluid from vessel 60 and then elution is initiated either by applying a single eluant from vessel 62 or using gradient elution through 70 gradient former.

Polishing purification of the product, obtained by continuous, immunoaffinity purification of secreted protein can also be accomplished as described in paragraphs 1, 2 and 3. These features allow the utilization of the instrument for the integrated production and purification of secreted product from cell culture with the built-in flexibility of applying a number of different purification strategies for product recovery with the objective of obtaining pure protein product while minimizing changes in product identity.

We claim:

1. An integrated cell culture and purification system for producing purified cell culture products, comprising:

a. a cell culture subunit for culturing cells, whereby said cells can secrete said product into a culture fluid;

b. a purification subunit linked to the cell culture subunit adapted to remove said product from said culture fluid; and

c. means for circulating said culture fluid from said cell culture subunit to said purification subunit.

2. The system of claim 1, wherein said system further includes means for discontinuing said circulation of cell culture fluid through said purification subunit and eluting purified product from said purification subunit

3. The system of Claim 1 wherein said cells are separated from the purification subunit by a membrane.

4. The system of Claim 1, wherein said cell culture system comprises a bioreactor for permitting contact between said cells and said culture fluid while preventing said cells from leaving said bioreactor when said culture fluid is circulated to said purification subunit.

5. The system of Claim 4, wherein said bioreactor is of hollow fiber type, and said cells are retained inside said hollow fibers.

6. The system of Claim 1, wherein said cell culture subunit further comprises an oxygenator for supplying oxygen to said cells by oxygenating said culture fluid.

7. The system of Claim 1, wherein said purification subunit comprises self-contained plug-in cartridge adapted for ready attachment to or removal from said system.

8. The system of Claim 1, wherein said bioreactor subunit comprises self-contained plug-in type bioreactor cartridge adapted for easy attachment to or removal from said system.

9. The system of Claim 1, wherein said bioreactor subunit comprises a thermostated culture medium vessel to maintain optimal temperature for cell culture.

10. The system of Claim 1, wherein said bioreactor subunit comprises a gas flow regulator to provide adequate oxygenation for the cell culture.

11. The purification subunit of Claim 7, wherein said cartridge comprises an activated medium for covalent attachment of proteins/ligands.

12. The activated medium of Claim 11, wherein said medium comprises aldehyde functional groups.

13. The activated medium of Claim 11, wherein said activated medium is sterilizable by autoclaving or sodium hydroxide.

14. The purification subunit of Claim 7, wherein said activated medium is derivatized with nonproteinaceous molecules.

15. The purification subunit of Claim 7, wherein said activated medium is derivatized with proteins.

16. The purification subunit of Claim 7, wherein said activated medium is derivatized with antibodies.

17. The purification subunit of Claim 7, wherein said activated medium is derivatized with light chain specific antibodies.

18. The purification subunit of Claim 7, wherein said activated medium is derivatized with heavy chain specific antibodies.

19. The purification subunit of Claim 7, wherein said activated medium is derivatized with antibodies at a concentration of 1mg/ml to 10 mg/ml resin.

20. The purification subunit of Claim 7, wherein said protein is coupled to said activated medium in said purification cartidge in such a way that said protein is injected onto said cartridge through a sterile port.

21. The system of Claim 1, wherein said purification subunit adsorbs secreted product from the cell culture medium.

22. The system of Claim 1, wherein said secreted product is a protein.

23. The secreted protein of Claim 22, wherein said secreted protein is a monoclonal antibody.

24. The system of Claim 1, wherein said secreted proteins are recovered from said purification cartridge in the steps of:

a, first, extensively washing the cartridge with a wash buffer to remove contaminating proteins;

b, eluting the cartridge with a mild elution medium to recover adsorbed protein;

c, regenerating the cartridge by consecutive washes with wash buffer and cell culture medium for the subsequent adsorption cycle.

25. The system of Claim 1, wherein said purified proteins are quantitated by ultra-violet spectrophotometry and subsequent integration of the protein peak.

26. The system of Claim 1, wherein the adsorption of said secreted protein is a continuous process.

27. The system of Claim 1, wherein the adsorption of said secreted protein is a discontinuous process.

28. The system of Claim 1, wherein said discontinuous adsorption process for said secreted proteins comprises the following steps of:

a. withdrawing spent cell culture medium from said bioreactor subunit by using a pump;
b. directing said spent medium onto said purification cartridge comprising an adsorption medium;
c. removing unbound cell culture medium constituents by a wash process;
d. recovering bound product by elution from said purification cartridge.

29. The discontinuous adsorption process of Claim 28, wherein said purification cartridge comprises an ion exchange medium.

30. The discontinuous adsorption process of Claim 28, wherein said purification cartridge comprises a hydrophobic interaction chromatography medium.

31. The discontinuous adsorption process of Claim 28, wherein said purification cartridge comprises an affinity chromatography medium.

32. The discontinuous adsorption process of Claim 28, wherein said product recovery process involves changing the ionic strength and/or the pH in the purification cartridge.

33. The system of Claim 1, wherein said system is equipped with a refrigeration unit to maintain the bioactivity of perishable materials.

34. The system of Claim 1, wherein said purified cell culture product is kept refrigerated.

35. The system of Claim 1, wherein said waste fluids exit the system through sterile filter ports.

36. The system of Claim 1, wherein said system is sterilized.

37. The sterilization method of Claim 36, wherein said sterilization is carried out by using the chemical sterilant, glutaraldehyde.

38. The chemical sterilant of Claim 37, wherein said sterilant is used at a concentration range of 2-15% (v/v).

39. The system of Claim 1, wherein said system is sterilized by autoclaving.

40. The system of Claim 1, wherein said system is set up for the production run by attaching the bioreactor and purification cartridges to the sterile system.

41. The system of Claim 1, wherein said bioreactor and purification cartridges are presterilized before connection to the system.

42. The system of Claim 1, wherein said purification cartridge comprises protein binding ligand.

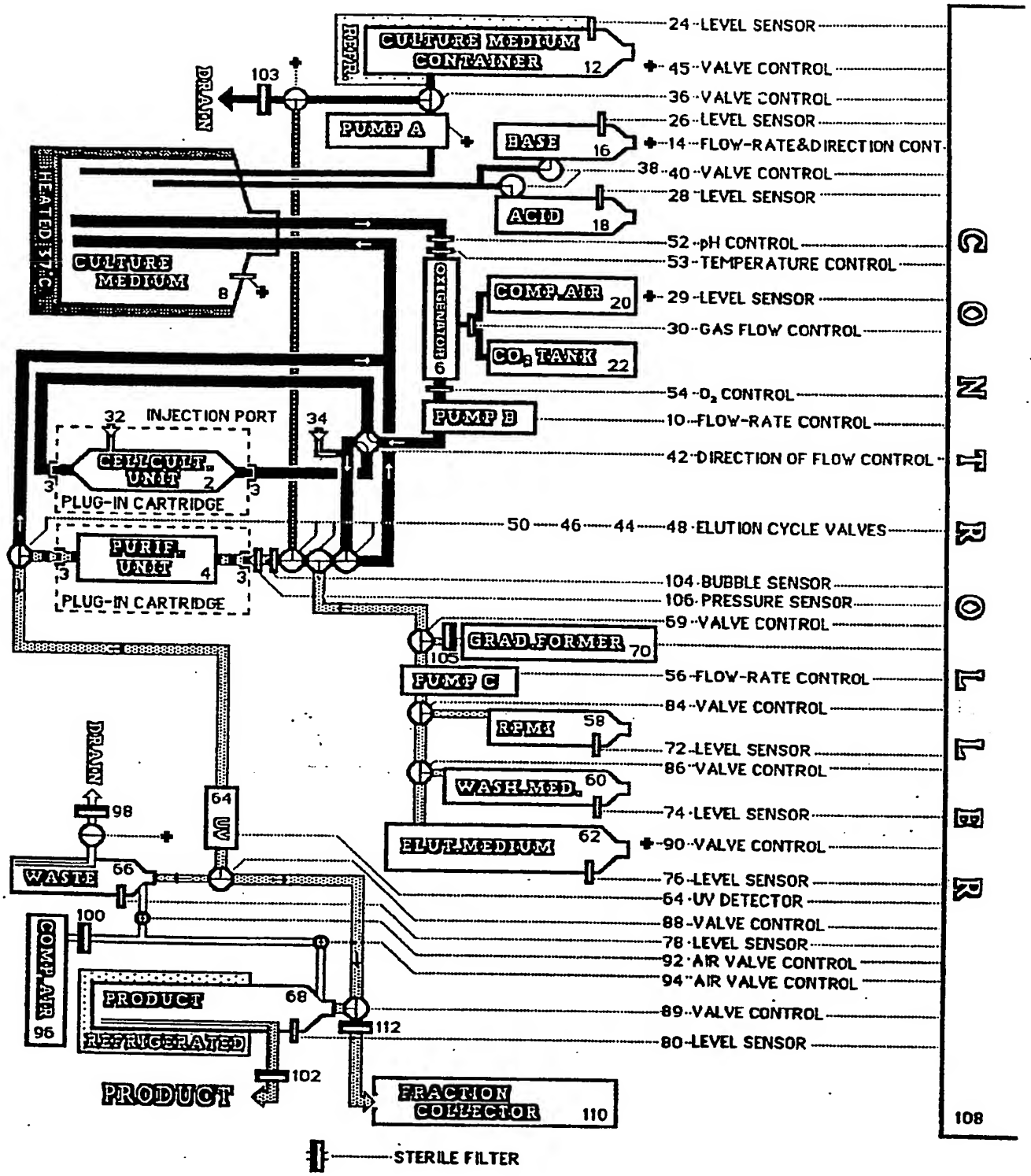
43. The system of Claim 1, wherein said protein binding ligand is an antibody.

44. The system of Claim 1, wherein said protein binding ligand is an ion exchange group.

45. The system of Claim 1, wherein said protein binding ligand is a hydrophobic group.

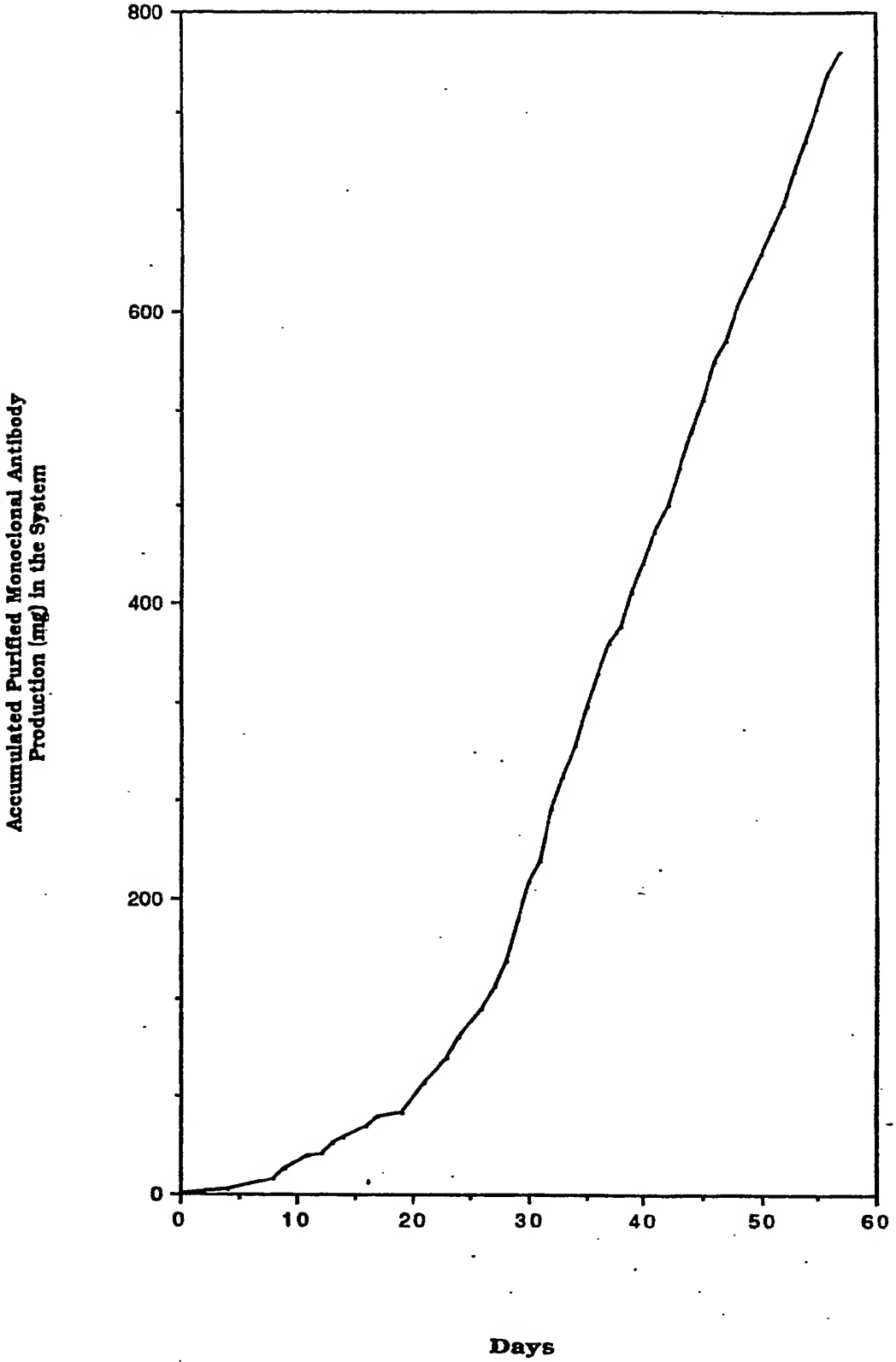
46. The system of Claim 1, wherein said protein binding ligand is an affinity ligand.

Figure 1



The Integrated Cell Culture Purification System

Figure 2


800
600
400
200
0
0
10
20
30
40
50
60
Accumulated Purified Monoclonal Antibody Production (mg) in the System
Days

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/04361

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) -

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C12 M 3/06
U.S. Cl: 435/284

II FIELDS SEARCHED

Minimum Documentation Searched 4

Classification System	Classification Symbols
U.S. CL.	435/69.1. 70.1, 284, 285,286,311,803,813; 210/679,196;263,282, 927; 530/412,413

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 5

III. DOCUMENTS CONSIDERED TO BE RELEVANT 14

Category *	Citation of Document, 16 with indication, where appropriate, of the relevant passages 17	Relevant to Claim No. 18
X Y	US,A,4,537,860 (TOLBERT ET AL.) 27 AUGUST 1985, see entire document.	1,3,4,6,21-28, 32,36-41 2,5,7-20,29-31, 33-35,42-46
Y	US,A,3,883,393 (KNAZEK ET AL.) 13 MAY 1975, see entire document.	5
A	US, A, 4,490,290 (GANI ET AL) 25 DECEMBER 1984, see entire document.	7,26,27,28
A	EP, A, 0,046,915 (NYLEN) 10 MARCH 1982, see entire document.	1-46

* Special categories of cited documents: 15

"A" document defining the general state of the art which is not considered to be of particular relevance

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search 2	Date of Mailing of this International Search Report 2
07 NOVEMBER 1990	11 JAN 1991
International Searching Authority 1	**Signature of Authorized Officer** 20
ISA/US	WILLIAM H. BEISNER

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